Amendments to the Specification

Please replace the paragraph disclosing priority information beginning on the first line of page 1 of the specification with the immediately following amended paragraph:

--The present application is a continuation-in-part of and claims priority under 35 U.S.C. § 120 to PCT/US99/28551 filed on December 2, 1999, which is a continuation-in-part of and claims priority under 35 U.S.C. § 120 to PCT/US99/28634 filed on December 1, 1999, which claims priority under 35 U.S.C. § 119 to U.S. Provisional Application Serial No. 60/119,537 filed February 10, 1999, now abandoned, which applications are incorporated herein by reference.--

Please replace the paragraph on page 35, lines 1-8 with the immediately following amended paragraph:

--The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (e.g., At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32} and radioactive isotopes of Lu), chemotherapeutic agents e.g. methotrexate, adriamycin™, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents, enzymes and fragments thereof such as nucleolytic enzymes, antibiotics, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof, and the various antitumor or anticancer agents disclosed below. Other cytotoxic agents are described below. A tumoricidal agent causes destruction of tumor cells.--

Please replace the paragraph on page 56, lines 12-20 with the immediately following amended paragraph:

--Preferred fusion partner myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a selective medium that selects against the unfused parental cells. Preferred myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California USA, and SP-2 and derivatives e.g., X63-Ag8-653 cells available from the American Type Culture Collection™, Manassas, Virginia, USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, J. Immunol., 133:3001 (1984); and Brodeur et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).--

Please replace the paragraph on page 86, lines 19-21 with the immediately following amended paragraph:

--Alternatively, or additionally, FISH assays such as the INFORM® (sold by Ventana™, Arizona) or PATHVISION® (Vysis™, Illinois) may be carried out on formalin-fixed, paraffin-embedded tumor tissue to determine the extent (if any) of TAT overexpression in the tumor.--

Please replace the paragraph on page 86, line 33 to page 87, line 17 with the immediately following amended paragraph:

--Currently, depending on the stage of the cancer, cancer treatment involves one or a combination of the following therapies: surgery to remove the cancerous tissue, radiation therapy, and chemotherapy. Anti-TAT antibody, oligopeptide or organic molecule therapy may be especially desirable in elderly patients who do not tolerate the toxicity and side effects of chemotherapy well and in metastatic disease where radiation therapy has limited usefulness. The tumor targeting anti-TAT antibodies, oligopeptides and organic molecules of the invention are useful to alleviate TAT-expressing cancers upon initial diagnosis of the disease or during relapse. For therapeutic applications, the anti-TAT antibody, oligopeptide or organic molecule can be used alone, or in combination therapy with, e.g., hormones, antiangiogens, or radiolabelled compounds, or with surgery, cryotherapy, and/or radiotherapy. Anti-TAT antibody, oligopeptide or organic molecule treatment can be administered in conjunction with other forms of conventional therapy, either consecutively with, pre- or post-conventional therapy. Chemotherapeutic drugs such as TAXOTERE® (docetaxel), TAXOL® (palictaxel), estramustine and mitoxantrone are used in treating cancer, in particular, in good risk patients. In the present method of the invention for treating or alleviating cancer, the cancer patient can be administered anti-TAT antibody, oligopeptide or organic molecule in conjuction with treatment with the one or more of the preceding chemotherapeutic agents. In particular, combination therapy with palictaxel and modified derivatives (see, e.g., EP0600517) is contemplated. The anti-TAT antibody, oligopeptide or organic molecule will be administered with a therapeutically effective dose of the chemotherapeutic agent. In another embodiment, the anti-TAT antibody, oligopeptide or organic molecule is administered in conjunction with chemotherapy to enhance the activity and efficacy of the chemotherapeutic agent, e.g., paclitaxel. The Physicians' Desk Reference™ (PDR) discloses dosages of these agents that have been used in treatment of various cancers. The dosing regimen and dosages of these aforementioned chemotherapeutic drugs that are therapeutically effective will depend on the particular cancer being treated, the extent of the disease and other factors familiar to the physician of skill in the art and can be determined by the physician.--

Please replace the paragraph on page 99, lines 28-31 with the immediately following amended paragraph:

--Commercially available reagents referred to in the examples were used according to manufacturer's instructions unless otherwise indicated. The source of those cells identified in the following examples, and throughout the specification, by ATCC accession numbers is the American Type Culture Collection™, Manassas, VA.--

Please replace the paragraph on page 99, line 34 to page 100, line 19 with the immediately following amended paragraph:

--An expressed sequence tag (EST) DNA database (LIFESEQ®, Incyte Pharmaceuticals™, Palo Alto, CA) was searched and interesting EST sequences were identified by GEPIS. Gene expression profiling *in silico* (GEPIS) is a bioinformatics tool developed at Genentech, Inc. that characterizes genes of interest for new cancer therapeutic targets. GEPIS takes advantage of large amounts of EST sequence and library information to determine gene expression profiles. GEPIS is capable of determining the expression profile of a gene based upon its proportional correlation with the number of its occurrences in EST databases, and it works by integrating the LIFESEQ® EST relational database and Genentech proprietary information in a stringent and statistically meaningful way. In this example, GEPIS is used to identify and cross-validate novel tumor antigens, although GEPIS can be configured to perform either very specific analyses or broad screening tasks. For the initial screen, GEPIS is used to identify EST sequences from the LIFESEQ® database that correlate to expression in a particular tissue or tissues of interest (often a tumor tissue of interest). The EST sequences identified in this initial screen (or consensus sequences obtained from aligning multiple related and overlapping EST sequences obtained from the initial screen) were then subjected to a screen intended to identify the presence of at least one transmembrane domain in the encoded protein. Finally, GEPIS was employed to generate a complete tissue expression profile for the various sequences of interest. Using this type of screening bioinformatics, various TAT polypeptides (and their encoding nucleic acid molecules) were identified as being significantly overexpressed in a particular type of cancer or certain cancers as compared to other cancers and/or normal non-cancerous tissues. The rating of GEPIS hits is based upon several criteria including, for example, tissue specificity, tumor specificity and expression level in normal essential and/or normal proliferating tissues. The following is a list of molecules whose tissue expression profile as determined by GEPIS evidences high tissue expression and significant upregulation of expression in a specific tumor or tumors as compared to other tumor(s) and/or normal tissues and optionally relatively low expression in normal essential and/or normal proliferating tissues. As such, the molecules listed below are excellent polypeptide targets for the diagnosis and therapy of cancer in mammals.--

Please replace the paragraph on page 100, lines 24-36 with the immediately following amended paragraph:

--A proprietary database containing gene expression information (GeneExpress®, Gene Logic™ Inc., Gaithersburg, MD) was analyzed in an attempt to identify polypeptides (and their encoding nucleic acids) whose expression is significantly upregulated in a particular tumor tissue(s) of interest as compared to other tumor(s) and/or normal tissues. Specifically, analysis of the GeneExpress® database was conducted using either software available through Gene Logic™ Inc., Gaithersburg, MD, for use with the GeneExpress® database or with proprietary software written and developed at Genentech, Inc. for use with the GeneExpress® database. The rating of positive hits in the analysis is based upon several criteria including, for example, tissue specificity, tumor specificity and expression level in normal essential and/or normal proliferating tissues. The following is a list of molecules whose tissue expression profile as determined from an analysis of the GeneExpress® database evidences high tissue expression and significant upregulation of expression in a specific tumor or tumors as compared to other tumor(s) and/or normal tissues and optionally relatively low expression in normal essential and/or normal proliferating tissues. As such, the molecules listed below are excellent polypeptide targets for the diagnosis and therapy of cancer in mammals.--

Please replace the paragraph on page 103, lines 4-22 with the immediately following amended paragraph:

--Parental BPH1 and BPH1CAFTD cells were maintained in RPMI1640 plus 5% fetal bovine serum (Gibco-BRL) in 5% CO_2 humidified tissue culture incubator. Cells were grown to 90% confluency in 100 mm tissue culture dishes (Falcon). Total RNAs were isolated using RNeasy columns (Qiagen™), treated with DNase I (Roche Molecular (BMB)) for 40 minutes at room temperature, and cleaned up by using RNeasy columns (Qiagen™). Oligonucleotide microarray experiments were performed and analyzed as previously described (Jin et al., Genentech, Inc.). Briefly, after being tested for quality on test arrays, the samples were hybridized for 16 hours at 45°C to Human Genome arrays (U95A, B, C, D, E). The arrays were washed, then stained with streptavidin-phycoerythrin (Genome arrays were amplified with an anti-streptavidin antibody). The arrays were scanned with the GeneArray™ scanner (Agilent™ Technologies). Raw data were collected and analyzed using Affymetrix™ Microarray Suite and Data Mining Tools software. Experiments were done in six replicates for two BPH1 samples. Mann-Whitney pair-wised comparison was performed to identify genes that were differentially expressed. Each of the six parental BPH1 samples, was compared to BPH1CAFTD samples resulting in 36 pairwise comparisons for both sample comparisons. Genes with the concordance exceeded 80.6% were considered as statistically significant ($p<0.05$). Gene lists from both comparisons were then loaded into GeneSpring™ software (Silicon Genetics, Redwood City CA) to look for common Affymetrix probe sets in both lists. This resulted in 493 probe sets increased in common to both lists and 398 in common that decreased expression relative to BPH1CAFTD. The expression of these genes was then looked at by examining human prostate adenocarcinomas and nodular hyperplasias compared to normal prostates in silico using the GeneLogic™ GeneExpress™ 2000 database.--

Please replace the paragraph on page 105, lines 4-14 with the immediately following amended paragraph:

--*E. coli* paste from 0.5 to 1 L fermentations (6-10 g pellets) is resuspended in 10 volumes (w/v) in 7 M guanidine, 20 mM Tris, pH 8 buffer. Solid sodium sulfite and sodium tetrathionate is added to make final concentrations of 0.1M and 0.02 M, respectively, and the solution is stirred overnight at 4°C. This step results in a denatured protein with all cysteine residues blocked by sulfitolization. The solution is centrifuged at 40,000 rpm in a Beckman Ultracentifuge for 30 min. The supernatant is diluted with 3-5 volumes of metal chelate column buffer (6 M guanidine, 20 mM Tris, pH 7.4) and filtered through 0.22 micron filters to clarify. The clarified extract is loaded onto a 5 ml Qiagen™ Ni-NTA metal chelate column equilibrated in the metal chelate column buffer. The column is washed with additional buffer containing 50 mM imidazole (Calbiochem, Utrol grade), pH 7.4. The protein is eluted with buffer containing 250 mM imidazole. Fractions containing the desired protein are pooled and stored at 4°C. Protein concentration is estimated by its absorbance at 280 nm using the calculated extinction coefficient based on its amino acid sequence.--

Please replace the paragraph on page 107, lines with the immediately following amended paragraph:

--Twelve micrograms of the desired plasmid DNA is introduced into approximately 10 million CHO cells using commercially available transfection reagents Superfect® (Qiagen™), Dosper® or Fugene® (Boehringer Mannheim). The cells are grown as described in Lucas et al., supra. Approximately 3 x 10^7 cells are frozen in an ampule for further growth and production as described below.--

Please replace the paragraph on page 108, lines 3-8 with the immediately following amended paragraph:

--For the poly-His tagged constructs, the proteins are purified using a Ni-NTA column (Qiagen™). Before purification, imidazole is added to the conditioned media to a concentration of 5 mM. The conditioned media is pumped onto a 6 ml Ni-NTA column equilibrated in 20 mM Hepes, pH 7.4, buffer containing 0.3 M NaCl and 5 mM imidazole at a flow rate of 4-5 ml/min. at 4°C. After loading, the column is washed with additional equilibration buffer and the protein eluted with equilibration buffer containing 0.25 M imidazole. The highly purified protein is subsequently desalted into a storage buffer containing 10 mM Hepes, 0.14 M NaCl and 4% mannitol, pH 6.8, with a 25 ml G25 Superfine (Pharmacia) column and stored at -80°C.--

Please replace the paragraph on page 109, lines 10-14 with the immediately following amended paragraph:

--Recombinant baculovirus is generated by co-transfecting the above plasmid and BaculoGold™ virus DNA (Pharmingen) into *Spodoptera frugiperda* ("Sf9") cells (ATCC CRL 1711) using Lipofectin™ (commercially available from GIBCO-BRL). After 4-5 days of incubation at 28°C, the released viruses are harvested and used for further amplifications. Viral infection and protein expression are performed as described by O'Reilley et al., Baculovirus expression vectors: A Laboratory Manual, Oxford: Oxford University Press (1994).--

Please replace the paragraph on page 109, lines 15-29 with the immediately following amended paragraph:

--Expressed poly-his tagged TAT can then be purified, for example, by Ni^{2+}-chelate affinity chromatography as follows. Extracts are prepared from recombinant virus-infected Sf9 cells as described by Rupert et al., Nature, 362:175-179 (1993). Briefly, Sf9 cells are washed, resuspended in sonication buffer (25 mL Hepes, pH 7.9; 12.5 mM $MgCl_2$; 0.1 mM EDTA; 10% glycerol; 0.1% NP-40; 0.4 M KCl), and sonicated twice for 20 seconds on ice. The sonicates are cleared by centrifugation, and the supernatant is diluted 50-fold in loading buffer (50 mM phosphate, 300 mM NaCl, 10% glycerol, pH 7.8) and filtered through a 0.45 μm filter. A Ni^{2+}-NTA agarose column (commercially available from Qiagen™) is prepared with a bed volume of 5 mL, washed with 25 mL of water and equilibrated with 25 mL of loading buffer. The filtered cell extract is loaded onto the column at 0.5 mL per minute. The column is washed to baseline A_{280} with loading buffer, at which point fraction collection is started. Next, the column is washed with a secondary wash buffer (50 mM phosphate; 300 mM NaCl, 10% glycerol, pH 6.0), which elutes nonspecifically bound protein. After reaching A_{280} baseline again, the column is developed with a 0 to 500 mM Imidazole gradient in the secondary wash buffer. One mL fractions are collected and analyzed by SDS-PAGE and silver staining or Western blot with Ni^{2+}-NTA-conjugated to alkaline phosphatase (Qiagen™). Fractions containing the eluted His_{10}-tagged TAT are pooled and dialyzed against loading buffer.--

Please replace the paragraph on page 111, lines 13-14 with the immediately following amended paragraph:

--The following materials have been deposited with the American Type Culture Collection™, 10801 University Blvd., Manassas, VA 20110-2209, USA (ATCC):--